Geographic basis of genetic variation in *Platymantis corrugatus*

By

Kerry Allen Cobb

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Chairperson Dr. Rafe Brown

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Dr. Leo Smith

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Dr. Rob Moyle

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The Thesis Committee for Kerry Allen Cobb
certifies that this is the approved version of the following thesis:

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ABSTRACT

The species *Platymantis corrugatus*—part of a highly diverse, endemic radiation of frogs in the Philippine archipelago—has an improbably widespread distribution that is without precedent in *Platymantis*. In this study, I estimate the phylogenetic relationships among individuals from across the range of *P. corrugatus* using maximum likelihood and Bayesian inference with multilocus DNA sequence data and analyze male advertisement call data to determine if patterns of diversification in *P. corrugatus* conform to predictions made by a prevailing model of diversification in the Philippines. In light of these data, I consider if unrecognized diversity is likely to be found within this putative species complex. The data largely support the prevailing model of diversification although there are some striking deviations that have important implications regarding the process of diversification of *P. corrugatus* and within other Philippine herpetofauna more generally. These data also suggest that *P. corrugatus* is composed of four distinct lineages with unique evolutionary histories and trajectories that each warrant recognition as species. The relationships between these putatively new species reveal a wide disjunction without precedent in the Philippines between the clade from the island of Mindoro and the clade from Camiguin Norte suggesting the possibility of a history of extinction or displacement by migrants from the Luzon Mindanao clade.

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INTRODUCTION

Frogs of the genus *Platymantis* (family Ceratobatrachidae) are by far the most diverse radiation of anurans in the Philippine Archipelago. Currently, 31 species are recognized (AmphibiaWeb, 2016) but an additional 35–40 putative species await description and it is likely additional taxa will be identified as efforts to survey Philippine biodiversity continue (Brown and Diesmos 2002; Brown et al. 2013; 2015). Larvae of *Platymantis* undergo direct development and emerge from eggs laid in terrestrial environments as small frogs—foregoing the familiar tadpole stage (Alcala 1962). This reproductive mode has allowed *Platymantis* to occupy habitats not suitable for many other anuran groups—any that undergo a larval stage requiring standing water (Brown and Alcala 1982)—such as small arid islands, limestone outcrops, and high elevation forests (Brown et al. 2015). The colonization of a wide variety of habitats likely played a significant role in shaping the patterns of diversity and distribution in the genus. But these patterns are also inextricably linked to the processes that generated the geographic template (Lomolino et al. 2010).

The islands of the Philippine Archipelago—straddling two tectonic plates and lying on the ring of fire—owe their origins to an extraordinarily complex geologic history (Aurelio et al. 2013). The archipelago's existence began with the formation of island arcs resulting from subduction of the. Philippine Sea oceanic Plate beneath the Eurasian continental plate some time in the Cretaceous 70–140 million years ago (Hall 2002). Subduction of the Philippine Sea Plate and island arc formation continued along various parts of this plate boundary into the Cenozoic—70 million

years ago to present. As the oceanic plate scraped beneath the less dense continental plate, it generated ophiolitic terranes, accreted already existing island fragments, and spawned volcanic activity—all the while increasing the land area of the archipelago (Hall 2002). The majority of island formation occurred in the Cenozoic, continuing up until approximately 5 or 10 million years ago at which point the islands looked much like they do today (Lomolino et al. 2010; Aurelio et al. 2013). These events, for which the details are poorly known, were responsible for the formation of the majority of islands in the archipelago, which are referred to collectively as the Philippines Mobile Belt (Aurelio et al. 2013). Also part of the archipelago is the Palawan–Mindoro Continental block composed of Palawan and Mindoro as well as a small Northern section of Panay (Yumul et al. 2008). The Palawan–Mindoro Continental block broke away from the Eurasian continental plate 40–35 million years ago, drifted Southeast, and collided with the Philippine Sea plate 12–15 million years ago (Aurelio et al. 2013).

It is unclear how and when *Platymantis* first colonized the Philippines. Molecular dating estimates put the split of *Platymantis* and *Cornufer*—the sister genus to *Platymantis*—somewhere around 50 million years ago (Wiens et al. 2009). It is likely that early evolution in *Platymantis* was driven by dynamics of island arc formation. As sea levels throughout the Cenozoic remained higher than they are today (Lomolino et al. 2010), dispersal must have played an important role. It certainly did for *Cornufer*, which reached distant islands in the Southwest Pacific (Brown et al. 2015). The Ceratobatrachidae are one of few groups with a distribution spanning Wallace's line and with near equal levels of diversity on either

side (Brown et al. 2015). As the details of island arc formation remain poorly understood, so to do the details of events leading to current patterns of diversity and distribution in *Platymantis* (Brown 1997).

Far more is understood regarding the more recent Pleistocene epoch, lasting 2.5 million years ago until about 12,000 years ago (Lomolino et al. 2010). During this time, it is not believed that any significant movement of tectonic elements took place as these seem to have been largely stationary for 5–10 million years (Hall 2002). This epoch, however, is marked by dramatic, periodic shifts in sea level brought about by large fluctuations in temperature and subsequent glaciation. Sea levels fell as much as 120m below current levels during glacial periods and rose again as high as current level during interglacial periods. As a consequence of these fluctuations, many islands—previously separated by considerable expanses of ocean—were joined into larger island complexes referred to as Pleistocene Aggregate Island Complexes (PAICs). This process of repeated PAIC formation has been hypothesized to have been the primary driver of diversity and distributions in the Philippines during the Pleistocene (Inger 1954; Leviton 1963) leading to the expectation that lineages within PAICs will be monophyletic in relation to lineages on other PAICs (Siler et al. 2010b; 2011). It is also expected that lineages on islands within PAICs will be monophyletic with respect to other islands in the same PAIC. Although several exceptions to the patterns leading to these predictions are now known (i.e., over water dispersal) it is generally considered to be important in many groups (Siler et al. 2010b; 2011).

Frogs of the genus *Platymantis* tend to have restricted ranges corresponding to single islands (Brown et al. 2015), adjacent pairs of mountains (Alcala et al. 1998) (Brown et al. 1999), limestone outcrops (Siler et al. 2009; 2010a), and even within narrow elevational ranges on single mountains (Brown and Carlos Gonzalez 2007). In fact all but three currently recognized species of *Platymantis* are restricted to a single PAIC or even smaller area (Brown et al. 2015). These exceptions are *P. corrugatus*, *P. guentheri*, and *P. dorsalis*. As it is currently recognized, *P. corrugatus* has a distribution encompassing nearly the entire archipelago. This species is known from islands of the Luzon, Mindano, Mindoro, and West Visayan PAICs as well as from the small isolated island of Camiguin Norte.

Over the past 25 years and with increasing biotic inventory work aimed at estimating true amphibian species diversity in the Philippines (Brown and Diesmos 2009) investigators have observed audibly discernible differences in calls from across this range and on the island of Camiguin Norte they are found on climbing on limestone outcrops as opposed to being found in leaf litter as is typical across the remainder of its distribution (Rafe M. Brown *pers. comm.*). Given these observations, and to the species' improbably widespread (biogeographically nonsensical) distribution, it is likely that *P. corrugatus* contains currently unrecognized species diversity. The proposition of multiple species in this group is not new. In fact Günther (1858) named *Platymantis plicifera* several years after Dumeril's (1853) description of *Hylodes corrugatus*, and both were recognized, albeit variably, until Peters (1873) and Boulenger (1882) placed *P. plicifera* in synonymy with *P. corrugatus*; this action was followed by Inger (1954) in his comprehensive review of

Philippine amphibians and has not been questioned since (Brown and Alcala 1970; Alcala 1986; Alcala and Brown, 1999).

In this study I examine the evolutionary relationships, population structure, and biogeography within the putative species complex *Platymantis corrugatus* using maximum likelihood and Bayesian inference phylogenetic estimates from multilocus sequence data along with bioacoustics data (male advertisement calls). With these data I address the following questions: (1) Does putative species diversity in the *P. corrugatus* species complex conform to expectations purely derived from the PAIC model of diversification (i.e., do all individuals within a PAIC or within an island form monophyletic groups with respect to other PAICs or islands respectively)? (2) Is diversity within PAICs structured geographically? (3) Has there been divergence in advertisement call characteristics, and if so, do these differences conform to geographic and phylogenetic patterns and hypothesized species boundaries? (4) Does all of this evidence, taken together, lend support to the possibility of unrecognized species diversity within *P. corrugatus*?

MATERIALS AND METHODS

—Field Work, Sample Collection, and Specimen Preservation

Fieldwork was conducted at localities throughout the Philippine Archipelago between 1992 and 2012. Specimens were collected predominately at night by triangulating on the positions of vocalizing males or by incidental encounters of individuals (day and night) in the open on the forest floor. Specimens were euthanized in aqueous chloretone, dissected for tissue samples (liver—preserved in

95% ethanol or RNALater), fixed in 10% buffered formalin, and then transferred to 70% ethanol within 2 months. Specimens were deposited in United States and Philippine museum collections (Appendix 2).

—Taxon Sampling Strategy and Outgroup Selection

I sequenced mitochondrial DNA from 196 specimens of *Platymantis corrugatus*, with representatives from nearly every locality from which tissues have been collected (Appendix 1). Additionally, mitochondrial DNA sequences were obtained from 17 outgroup samples (Appendix 2) representing other species from the genus *Platymantis*, two species from the sister genus *Ingerana* (Brown et al. 2015), and 4 distantly related ranid outgroup (*Pulchrana moellendorfii, Sangirana sanguinea, Hylarana nicobariensis,* and *Hoplobatrachus rugulosus*; (Wiens et al. 2009). Nuclear protein coding sequences from three loci were obtained from 48 individuals selected equitably across clades from the preliminary mtDNA maximum likelihood topology including five of the outgroup taxa (Appendix 2).

—DNA Extraction, Purification, and Amplification

I extracted genomic DNA from liver tissues using Fujita's guanidine thiocyanate protocol, as described by (Esselstyn et al. 2008). I amplified DNA, with polymerase chain reaction (PCR), an approximately 900 base pair portion of the 16S mitochondrial rRNA gene using the primers 16Sc–L and 16Sd–H from (Evans et al. 2003) and 900, 1050, and 770 base pairs from the CPT2, DNAH3, and LCT nuclear protein coding loci respectively using the nested primers and protocol of (Shen et al. 2013). Amplification was confirmed by visualizing PCR product on 1% agarose gel. PCR products were purified with ExoSAP–IT (US78201, Amersham Biosciences,

Piscataway, NJ, USA). Purified PCR product was cycle sequenced using ABI Prism BigDye Terminator chemistry (Ver. 3.1; Applied Biosystems, Foster City, CA, USA). Cycle sequencing product was isolated using Sephadex (NC9406038, Amersham Biosciences) in Centri–Sep 96 spin plates (CS–961, Princeton Separations, Princeton, NJ, USA) and analyzed on an ABI Prism 3730 Genetic Analyzer (Applied Biosystems).

—Assembly, Alignment, and Phylogenetic Analyses

Trimming and *de novo* contig assembly of raw sequence reads was accomplished with Geneious Pro v5.3. Assembled CPT2, DNAH3, and LCT contigs were aligned using the MAFFT–auto algorithm (Katoh and Toh 2008) implemented in Geneious Pro v5.3 under default parameters. Due to substantial variation in the 16S mtDNA contig length, Ns were appended to the ends of contigs until each sequence was as long as the longest sequence. This prevented alignment of non–homologous regions that share sequence similarity leading to wildly inaccurate alignments. Assembled and buffered 16S contigs were aligned using the MAFFT–xinsi algorithm implemented in the stand alone Mafft v7.299 run under default parameters (Katoh and Toh 2008). Alignments of nuclear protein coding sequences and their amino acid translations were inspected visually in Geneious v5.3., and any bases that created gaps in the alignment and stop codons within the sequence were excluded from all downstream analyses based on the assumption that these bases are merely sequencing errors.

Phylogenetic tree topologies were independently estimated for each locus and for a concatenated dataset of all loci using maximum–likelihood (ML) and Bayesian inference (BI). The estimate of the 16S gene tree topology was used as a

guide (i.e., individuals for nDNA sequencing were selected from each major mtDNA haplotype clade) for reduced taxon sampling and sequencing of the nuclear protein coding loci. Optimal partitioning strategy and substitution models used in each phylogenetic tree estimate were determined with ParitionFinder v1.1.1 (Lanfear et al. 2012) using the Bayesian Information Criterion and unlinked branch lengths. A single partition was defined in PartitionFinder for the 16S locus and for nuclear protein coding loci a separate partition was defined for each codon position. The 'all' algorithm was implemented in PartitionFinder for estimating the optimal concatenated data partitioning scheme—searching over any combination of the aforementioned partitions.

Partitioned maximum likelihood analyses were performed in RAxML v8.1.20 (Stamatakis 2006) with 1000 search replicates, 1000 nonparametric bootstrap replicates, a random starting tree, and the partitioning scheme and substitution models (Table 1) determined by PartitionFinder. Partitioned Bayesian inferences were performed with MrBayes v3.2.4 (Ronquist et al. 2012) using the partitioning scheme and substitution models determined in PartitionFinder (Table 1). The relative substitution rate, gamma shape parameter, proportion of invariant sites, and character state frequencies were unlinked. Two independent runs of four MCMC incrementally heated chains (temperature = 0.2) were run for 20 million generations using default priors and were sampled every 1000 generations. I conservatively discarded the first 20% of samples as 'burn–in'.

—Acoustic Analysis of Advertisement Calls

Advertisement calls were recorded in the field using Sony™ WM DC6 Professional Walkman and a Sennheiser TM ME80 condenser microphone with K3U power module. Calls were recorded from a distance of 0.5–1 meter. Ambient air and frog cloacal temperatures—measured immediately after recording with a fast reading analog thermometer—are available for 12 out of a total of 17 recordings. Definitions used here follow Duellman and Trueb (1994) in defining a call as a series of notes, where notes are distinct segments punctuated temporally by a return to background noise. Pulsed notes are notes containing clear amplitude peaks as opposed to the relatively constant amplitude characteristic of tonal notes (Hutter et al. 2015). An individual pulse refers to a single amplitude peak within a pulsed note. Calls were analyzed in RavenPro v1.4 (Bioacoustics Research Program, 2011) and measurements of temporal and spectral characters were obtained from waveform (amplitude plotted as a function of time) and audiospectrograms (frequency plotted against time) plots. The spectrogram was computed in RavenPro with a short–time Fourier transform using a Hanning window with a width of 256 samples and 50 points of overlap. I measured or calculated the following variables: (1) dominant frequency (Hz); (2) center frequency (frequency [Hz] that divides note into two frequency intervals of equal energy); (3) bandwidth (Hz); (4) pulse number; (5) note duration (s); (6) time at peak amplitude (s); (7) pulse rate (/s) and (8) note envelope shape (time at peak amplitude / note duration). The range of values followed by the mean ± two standard deviations from the mean are reported for each variable (Table 2).

RESULTS

—Phylogeny and Population Structure

The final alignments for the 16S, CPT2, DNAH3, LCT, and the concatenated multilocus alignment contained 937, 809, 916, 708, and 3370 base pairs, respectively, and there were 679, 300, 188, 232, and 1504 informative sites in each alignment respectively (Table 1). For each alignment including the concatenated multilocus alignment, the highest ranked partitioning scheme from PartitionFinder was a single partition. See Table 1 for models of sequence evolution applied to each phylogenetic tree estimate.

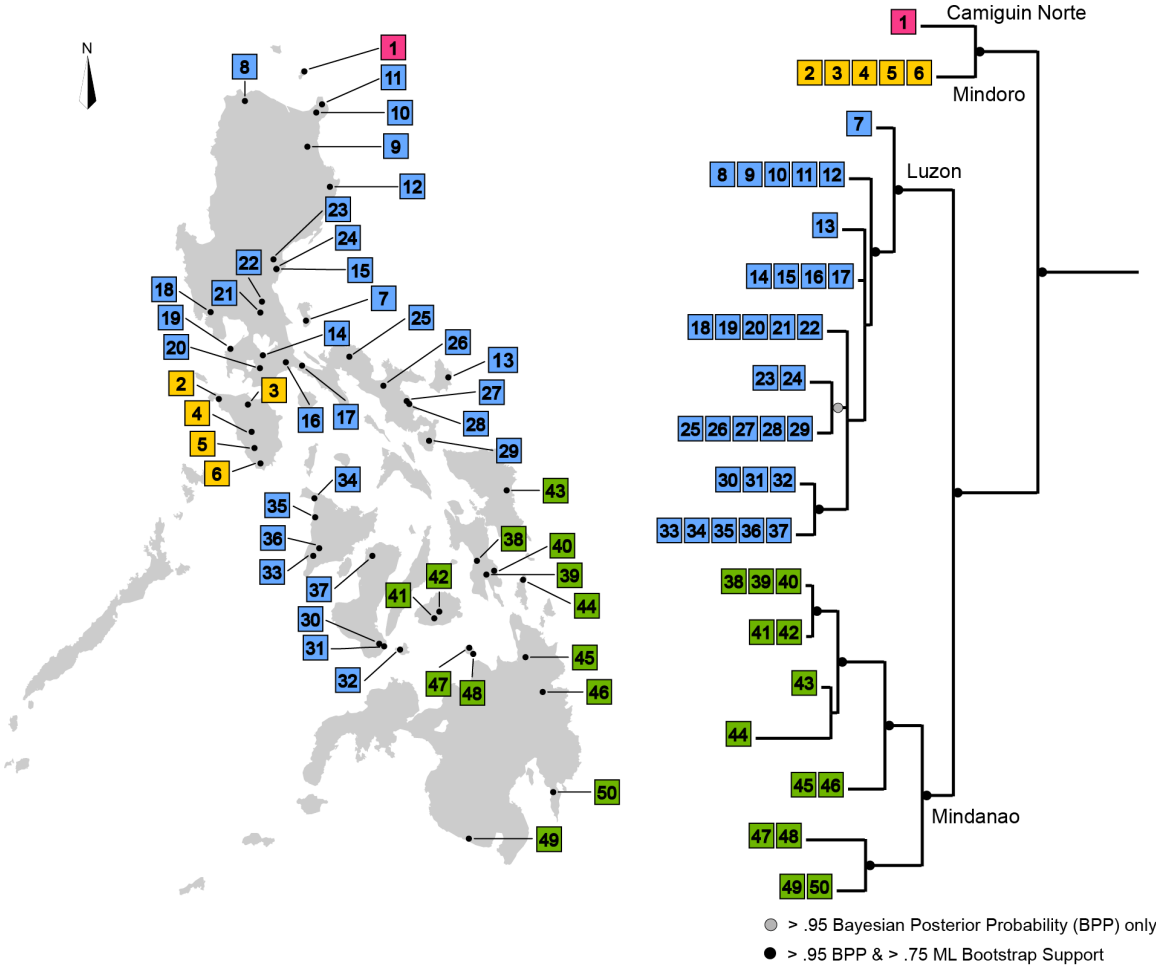
Table 1. Locus identities, sequence characteristics, and results of model selection procedure (see text for details).

Alignment	Best model inferred for RAxML	Best model inferred for MrBayes	Alignment Length	Parsimony Informative Sites
16S mtDNA	GTR+I+ Γ	GTR+I+ Γ	937	679
CPT2	GTR+ Γ	K80+ Γ	809	300
DNAH3	GTR+ Γ	HKY+ Γ	916	188
LCT	GTR+I+ Γ	K80+ Γ	708	232
Combined	GTR+I+ Γ	GTR+I+ Γ	3370	1399

There were no well-supported incongruences between ML or BI phylogenetic estimates from any of the alignments, nor were there any well-supported incongruences between loci. Given the absence of statistical support for character set partition incongruence, all data were concatenated into a single partitioned dataset (Table 1). The topology inferred from phylogenetic estimates of the concatenated data (Fig. 1) was used in subsequent considerations of geographical

distribution of genetic variation, comparisons to call data, and the inference of species boundaries. I observed high support for the monophyly of the *Platymantis corrugatus* Complex and highly supported phylogenetic structure, corresponding to major geographic features of the archipelago (Fig. 1). The first split in the phylogeny divides *P. corrugatus* into a clade composed of narrowly distributed phylogroups from the islands of Mindoro Island (central Philippines) and Camiguin Norte Island (extreme northern Philippines) versus a large more widely distributed clade, composed of two widely distributed phylogroups from the Luzon, West Visayan (central and northern Philippines and Mindanao (southern Philippines) PAICs, respectively. Within the Mindoro–Camiguin Norte clade there is a highly supported sister relationship between a clade of individuals from Mindoro Island (population numbers: 2–6, Fig. 1) and a Camiguin Norte clade (population number 1; Fig. 1). Similarly, within the Luzon–Visayan–Mindanao PAICs clade there is a highly supported split between a Luzon–West Visayan PAICs phylogroup and a Mindanao PAIC phylogroup. The individuals from the Visayan PAIC (30–37, Fig. 1) form a monophyletic group, however it is nested within the Luzon PAIC clade.

Figure 1. Phylogenetic relationships within *P. corrugatus* estimated with BI from concatenated mulitlocus sequence data. Tips represent highly supported (>0.95 BI posterior probability and > 0.75 ML bootstrap support) groups of individuals from multiple localities (numbered boxes).



Within the more widely distributed Luzon–West Visayan and Mindanao phylogroups, there are a number of highly supported clusters that conform to smaller geographic areas. There are seven well-supported divergent groups within the Mindanao clade corresponding to southern Mindanao (49–50, Fig. 1), northeast Mindanao (45–46, Fig. 1), Camiguin Sur Island (47–48, Fig. 1), Dinagat Island (44, Fig. 1), Bohol Island (41–42, Fig. 1), Leyte Island (38–40, Fig. 1), and Samar Island (43, Fig. 1). Within the Luzon PAIC clade there are nine well-supported divergent

groups. Two are nested within the monophyletic Visayan PAIC clade. One includes individuals from localities on Panay Island (33–36, Fig. 1) and from the northern locality on Negros Island (37, Fig. 1). The other distinct lineage occurs on the southern portion of Negros Island (30–32, Fig. 1) and on Siquijor Island (32, Fig. 1). There are seven divergent lineages on Luzon Island corresponding to northern Luzon (8–12, Fig. 1), Catanduanes (13, Fig. 1), southwest Luzon (14–17, Fig. 1), east Luzon (18–22, Fig. 1), Polillo Island(7, Fig. 1), the Bicol Peninsula (25–29, Fig. 1), and south Luzon (14–17, Fig. 1). The relationships between these divergent populations on Mindanao Island are well-supported whereas relationships between populations on Luzon are poorly- to moderately-supported with discordance at this level between trees estimated for each separate locus.

—Advertisement Calls

Recordings were obtained from 17 individuals in total from one, five, two, and eight individuals from Mindoro, Camiguin Norte, Mindanao, and Luzon islands respectively (Appendix 1). Samples representing the Luzon clade come from three separate localities—at the northern end of the Sierra Madres range, at the southern end of the Sierra Madres range, and from Mt. Isarog on the Bicol Peninsula. From each of the other major clades only a single locality is represented. The advertisement call of the *Platymantis. corrugatus* complex is pulsed; variable quality of recordings precluded counting of pulses for every recorded note. Pulses from at least some notes were successfully counted for every individual (Table. 2).

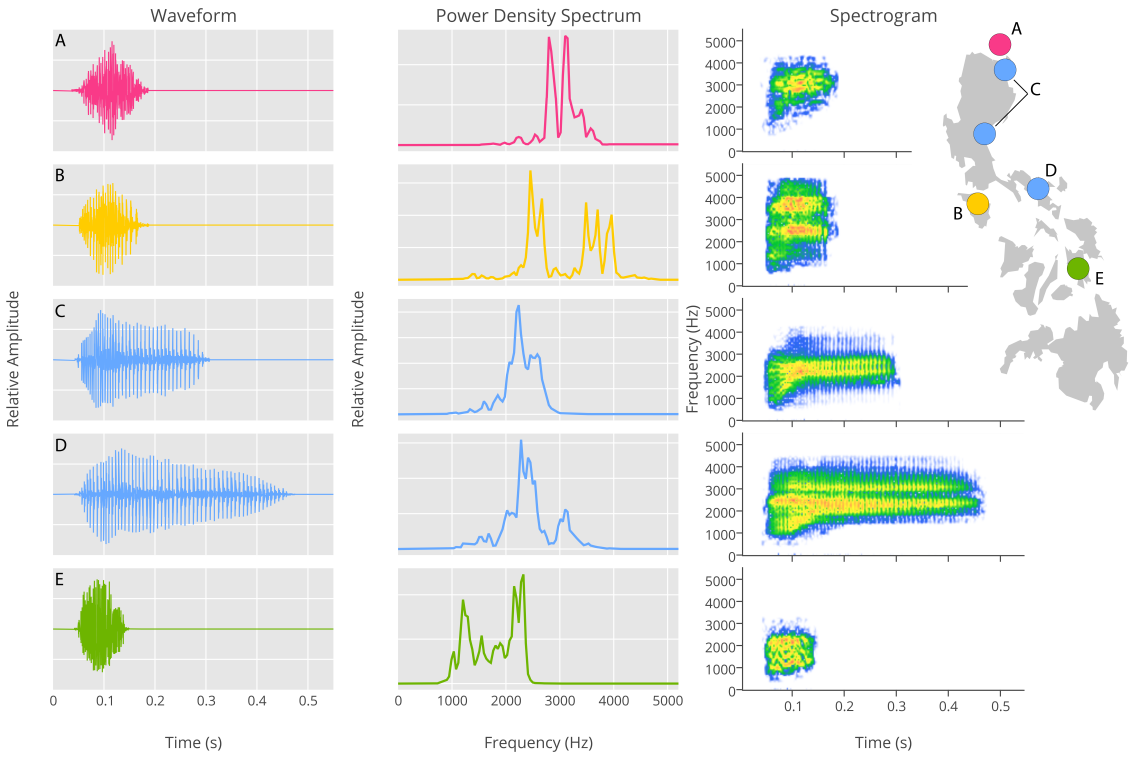
Table 2. Comparison of adverstisement calls recorded for the *Platymantis corrugatus* complex. Data are the range followed by the mean ± two standard deviations in parentheses.

Parameter	Camiguin Norte	Mindoro	Mindanao	Luzon–combined	Sierra Madres–Luzon	Bicol Peninsula (Luzon)
n-individuals	5	1	2	8	4	4
n-notes (range, total)	50–78, 326	69	37–66, 164	9–134, 355	22–134, 309	9–37, 46
Dominant frequency (Hz)	2411–3445 (2914±494)	2584–3789 (3160±708)	1205–2756 (2278±771)	1205–3100 (2557±592)	2067–3100 (2599±561)	1205–2756 (2273±479)
Highest frequency (Hz)	3571–4639 (3977±294)	4055–5573 (4590±539)	2598–3920 (3346±891)	2765–5661 (3534±879)	2765–3672 (3384 ±345)	4020.–5661 (4545±684)
Lowest frequency (Hz)	622–1442 (10302±261)	0–1373 (822±453)	339–698 (559±161)	270–1209 (777±328)	445–1209 (810±283)	270–797 (553±250)
Note duration(s)	0.078–0.214 (0.132±0.025)	0.103–0.218 (0.138±0.050)	0.060–0.124 (0.094±0.027)	0.192–0.518 (0.280±0.098)	0.192–0.327 (0.263±0.032)	0.308–0.518 (0.396±0.065)
Peak time (s)	0.015–0.096 (0.050±0.033)	0.035–0.116 (0.056±0.042)	0.004–0.070 (0.035±0.032)	0.022–0.247 (0.077±0.092)	0.022–0.213 (0.078±0.093)	0.031–0.247 (0.066±0.082)
Pulse rate (/s)	185–384 (258±93)	123–212. (169±37)	218–500 (367±104)	123.–294 (194±85)	154–294 (208±71)	123–203 (140±30)
Note envelope	0.126–0.835 (0.381±0.245)	0.202–0.733 (0.409±0.255)	0.041–0.676 (0.373±0.327)	0.081–0.802 (0.280±0.343)	0.081–0.782 (0.297±0.344)	0.086–0.802 (0.169±0.244)
Pulse number	23–47 (33±11)	18–32 (24±6)	19–46 (35±10)	39–87 (55±20)	39–87 (55±21)	47–74 (55±15)

There are slight differences in call variables between individuals from the two sampled regions on Luzon: the Sierra Madres range and the Bicol Peninsula, with note duration tending to be longer in the Bicol Peninsula than in Sierra Madres calls. The calls from these two regions are otherwise very similar (Table 2). Dominant frequencies within the Mindoro-Camiguin Norte (M = 2957, SD=569) and Luzon-Mindanao (M = 2469, SD = 703) sister groups are very similar while between them there is a difference of approximately 500 Hz. All *Platymantis corrugatus* have

two major frequency components in a note with higher energy typical of the lower frequency band (Fig. 2). This is not true in the Mindoro clade where the opposite is the case (Fig. 2). I found substantial between-group differentiation in relative amplitude of each frequency component and call frequency itself differed among all four subclades (Fig. 2).

Figure 2. Comparative oscillograms (left), spectral density (center) and spectrograms (right) of single notes of the advertisement calls on the same time scale in the *Platymantis corrugatus* complex. Localities shown are: (A) Camiguin Norte; (B) Mindoro; (C)Sierra Madres Range, Luzon; (D) Bicol Peninsula, Luzon; and (E) Luzon combined. Spectral density and spectrogram plots were produced with a custom python script using a short–time Fourier transform with a Hanning window with a length of 1024 samples and 1000 points of overlap.



DISCUSSION

The PAIC model of diversification predicts that species ranges (Siler et al. 2010b; 2011) and by extension community assemblages (Heaney 1985) will be concordant with PAIC boundaries. An important expectation derived from this prediction is that individuals within PAICs are monophyletic with respect to individuals from others (Siler et al. 2010b). There are two ways in which a species might violate this model that should each manifest themselves in different ways in

estimates of evolutionary relationships. First, individuals from different PAICs may be polyphyletic or otherwise genetically homogenous suggesting a high degree of migration and admixture between PAICs. This might result from having a high over–water dispersal ability such as being able to fly or from being very tolerant of salt water (Heaney 1985). Alternatively, individuals from different PAICs may be paraphyletic in relation to one another due to incomplete lineage sorting or relatively recent dispersal events.

Although a number of studies have identified herpetofauna which do have ranges spanning PAIC boundaries, few have been confirmed to be widely distributed across many PAICs in the archipelago (Brown et al. 2010). If *Platymantis corrugatus* were truly a single, wide ranging, admixed species it would be the first evidence of such within *Platymantis* which tend to be very range restricted (Alcala et al. 1998; Brown et al. 1999; Brown and Carlos Gonzalez 2007; Siler et al. 2009; 2010a; Brown et al. 2015). The phylogenetic relationships estimated in this study do not support a scenario of purely PAIC driven diversification. Individuals from the Mindanao and Mindoro PAICS as well as from the isolated island of Camiguin Norte do form highly supported monophyletic groups corresponding to PAICs (Figure 1). However, individuals from the West-Visayan PAIC, are nested within the Luzon phylogroup resulting in a paraphyletic relationship between them. Although this pattern conflicts with the PAIC model, dispersal of herpetofauna between Luzon and the West Visayan PAIC is known to have occurred numerous times (Linkem et al. 2011; Siler et al. 2011).

The theoretical framework presented by Wilson and MacArthur (1967) regarding island biogeography predicts that diversity islands is dependent on both its size and distance from a source of dispersal. Camiguin Norte, a small isolated island approximately 40km off the North coast of Luzon, is separated from Luzon by a deep ocean channel and the two landmasses have never shared land bridge connections. The Camiguin Norte phylogroup must have colonized the island via overwater dispersal. The most obvious route for dispersal to Camiguin Norte would be from the adjacent island of Luzon. If *Platymantis. corrugatus* colonized Camiguin Norte from Luzon, individuals from Camiguin Norte should be sister to individuals from Luzon. However, the Camiguin Norte phylogroup is sister to the Mindoro phylogroup revealing an apparently disjunct distribution within the Mindoro–Camiguin Norte clade. Could Camiguin Norte have been colonized from Mindoro or vice versa? The position of Mindoro—part of the Palawan–Mindoro continental block—was once much farther to the North of Luzon before reaching its current position approximately 15 million years ago (Hall 2002). However the northern—and oldest—part of Camiguin Norte is composed of volcanic material from only 3–7 million years old (Defant et al. 1989). A more plausible scenario is that an ancestral population on Luzon, belonging to the Mindoro–Camiguin Norte clade has gone extinct following the colonization of Mindoro and Camiguin Norte. Following this extinction, the extant Luzon lineage might have then recolonized Luzon from elsewhere in the archipelago. The relationships within the Luzon phylogroup are consistent with a recent and rapid radiation (i.e. short internode branch lengths with poorly resolved relationships). Genome wide nuclear sequence data from

numerous additional loci may illuminate past demography within the Luzon phylogroup and allow for explicit testing of a hypothesis of recent radiation within the group. The findings here, which suggest the possibility of a recent extinction and recolonization, are to my knowledge, the first evidence of such an occurrence among Philippine herpetofauna. Few small islands, like Camiguin Norte, have been the focus of biodiversity inventories. Investigators interested in the processes of diversification in the Philippines may be well served by focusing future efforts on small peripheral islands not associated with PAICs which could potentially harbor relictual taxa.

Male advertisement calls in frogs have been shown to play an important role in species recognition during reproduction (Ryan and Rand 1993; Phelps et al. 2006). Discrimination by females is based on both spectral and temporal components of a call (Gerhardt et al. 2000). Both of these components differ among the *Platymantis corrugatus* phylogroups and are consistent across populations within at least one phylogroup (Luzon PAIC). Interestingly, the most striking difference between phylogroups is between individuals from the Bicol Peninsula and individuals from Leyte Island (Figure 2). This region has been recognized as an important dispersal corridor between Luzon and Mindanao (Brown et al. 2013). During the most dramatic glacial maximums, a land bridge likely formed across the San Bernardino strait—potentially facilitating migration between the two island complexes. There is widespread evidence of reproductive character displacement in anurans of both female mate choice (Gerhardt 1994) and male vocalization (Blaire 1974; Höbel and Gerhardt 2003). Additional recording data from unrepresented

localities and mate choice experiments will be necessary to test the hypothesis of reproductive character displacement between the Luzon and Mindanao phylogroups. Additional recording data will also be necessary to test the statistical significance of observed differences between phylogroups that were outlined above.

The strongly supported monophyly of the four separate phylogroups and the highly supported relationships among them suggest that these four lineages have been evolving in isolation for a considerable period of time. In this period of time, there has been diversification within phylogroups among geographically structured populations. Additionally, the male advertisement calls have become consistently—at least within the Luzon PAIC—differentiated among the phylogroups in ways that have been demonstrated to contribute to reproductive isolation in many anuran species. The phylogroup from Camiguin Norte inhabits limestone outcrops unlike other members of the *Platymantis corrugatus* complex which inhabit leaf litter on the ground. Association with limestone seems to be associated with having a highly restricted distribution. Several of the *Platymantis* limestone specialist are still only known from the type localities (Brown and Carlos Gonzalez 2007; Siler et al. 2009; 2010a). All told, the evidence presented here (Table 3) indicate that *P. corrugatus* likely represents a complex of four separate species with unique evolutionary trajectories that warrant recognition as such (De Queiroz 1996).

Table 3. Summary of evidence supporting unrecognized species diversity in *Platymantis corrugatus*.

	Camiguin Norte	Mindoro	Luzon	Mindanao
Phylogeny	Monophyletic with high support	Monophyletic with high support	Monophyletic with high support	Monophyletic with high support
Range	Restricted to single deep water island, widely disjunct distribution with its sister clade	Restricted to single PAIC, widely disjunct distribution with sister clade	Problematic. Paraphyletic relationship with West Visayan Islands. Yet not a close relationship with closer Mindoro and Camiguin Norte	Restricted to PAIC, highly structured populations indicates little if any ongoing migration
Bioacoustics	Narrow frequency spectrum	Wide frequency spectrum	Longer note duration than any others	Much shorter note than Luzon with which it has periodically shared a land bridge
Taxonomic History	None	None	Synonymized with historically recognized species from Mindanao	*Platymantis plicifera* from Mindanao synonomized with *P. corrugatus* from Luzon (Peters 1873)
Habitat	Limestone	Leaf Litter	Leaf Litter	Leaf Litter
Body Size	Smallest	Intermediate	Intermediate	Largest

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APPENDIX 1

Specimen voucher numbers, field numbers, geographic locality, mass of individual, cloacal temperature and ambient air temperature for each call recording. *No voucher data available

Institution Number	Field Number	Locality	Date Recorded	Mass (g)	Cloacal Temperature (°C)	Air Temperature (°C)
KU 321971	RMB 12192	Villa Aurora, Maria Aurora, Aurora, Philippines	30 May 2009	2.4	23	21.5
KU 322163	RMB 12394	Villa Aurora, Maria Aurora, Aurora, Philippines	30 May 2009	1.2	25.2	21
KU 304590	RMB 5710	Balatubat, Calayan, Cagayan, Philippines	5 Mar 2006	1.8	26.3	22.6
KU 304661	RMB 5781	Balatubat, Calayan, Cagayan, Philippines	8 Mar 2006	1.9	24.5	23.4
KU 304662	RMB 5782	Balatubat, Calayan, Cagayan, Philippines	8 Mar 2006	1.8	24.5	23.9
KU 304663	RMB 5783	Balatubat, Calayan, Cagayan, Philippines	8 Mar 2006	1.8	25.5	24.3
KU 304664	RMB 5784	Balatubat, Calayan, Cagayan, Philippines	8 Mar 2006	1.7	25.3	24.1
KU 7698	RMB 4338	Guadalupe, Baybay, Leyte, Philippines	28 Aug 2002	4.9	26.2	25
KU 7699	RMB 4341	Guadalupe, Baybay, Leyte, Philippines	28 Aug 2002	2.1	25.6	25.2
KU 61977	RMB 3330	Panicuason, Naga City, Camarines Sur, Philippines	26 Jul 2001	2.1	N/A	N/A
KU 61978	RMB 3331	Panicuason, Naga City, Camarines Sur, Philippines	26 Jul 2001	2.3	N/A	N/A
KU 62003	RMB 3416	Panicuason, Naga City, Camarines Sur, Philippines	2 Aug 2001	2.1	24.5	22.2
KU 61980	RMB 3417	Panicuason, Naga City, Camarines Sur, Philippines	2 Aug 2001	2.2	22.3	21.6
*	*	Magrafil, Gonzaga, Cagayan, Philippines	22 July 2011	N/A	N/A	N/A
*	*	Magrafil, Gonzaga, Cagayan, Philippines	N/A	N/A	N/A	N/A
*	*	na, na, Mindoro, Philippines	N/A	N/A	N/A	N/A

APPENDIX 2

Summary of specimens and respective loci sequenced in this study. Map column refers to localities in Figure 1.
Locality number for Figure 1, specimen voucher number, field number and geographic locality for specimens sequenced in this study.

			Locus					
Species	Map	Locality	16S	CPT2	DNAH3	LCT	Field ID	Institution ID
Platymantis corrugatus	1	Philippines, Cagayan, Calayan	x		x	x	RMB 5765	*
Platymantis corrugatus	1	Philippines, Cagayan, Calayan	x				RMB 7341	*
Platymantis corrugatus	1	Philippines, Cagayan, Calayan	x	x	x	x	RMB 7342	*
Platymantis corrugatus	2	Philippines, Occidental Mindoro, Paluan	x	x	x	x	ELR 790	KU 308350
Platymantis corrugatus	2	Philippines, Occidental Mindoro, Paluan	x				ELR 914	KU 308458
Platymantis corrugatus	2	Philippines, Occidental Mindoro, Paluan	x				ELR 915	KU 308459
Platymantis corrugatus	3	Philippines, Oriental Mindoro, San Teodoro	x				*	CMNH 806
Platymantis corrugatus	3	Philippines, Oriental Mindoro, San Teodoro	x	x	x	x	*	CMNH 809
Platymantis corrugatus	3	Philippines, Oriental Mindoro, San Teodoro	x				*	PMNH 806
Platymantis corrugatus	3	Philippines, Oriental Mindoro, San Teodoro	x	x	x	x	*	PMNH 809
Platymantis corrugatus	4	Philippines, Occidental Mindoro, Sablayan	x				ACD 1406	*
Platymantis corrugatus	4	Philippines, Occidental Mindoro, Sablayan	x	x	x	x	ACD 1410	*
Platymantis corrugatus	4	Philippines, Occidental Mindoro, Sablayan	x				ELR 278	*
Platymantis corrugatus	4	Philippines, Occidental Mindoro, Sablayan	x				ELR 360	*
Platymantis corrugatus	4	Philippines, Occidental Mindoro, Sablayan	x				ELR 363	*
Platymantis corrugatus	4	Philippines, Occidental Mindoro, Sablayan	x				ELR 366	*
Platymantis corrugatus	4	Philippines, Occidental Mindoro, Sablayan	x				ELR 376	*
Platymantis corrugatus	4	Philippines, Occidental Mindoro, Sablayan	x				ELR 377	*
Platymantis corrugatus	4	Philippines, Occidental Mindoro, Sablayan	x				ELR 419	*
Platymantis corrugatus	4	Philippines, Occidental Mindoro, Sablayan	x				ELR 425	*

Platymantis corrugatus	4	Philippines, Occidental Mindoro, Sablayan	x				ELR 426	*
Platymantis corrugatus	4	Philippines, Occidental Mindoro, Sablayan	x				ELR 427	*
Platymantis corrugatus	4	Philippines, Occidental Mindoro, Sablayan	x				ELR 529	*
Platymantis corrugatus	4	Philippines, Occidental Mindoro, Sablayan	x	x	x	x	ELR 622	*
Platymantis corrugatus	4	Philippines, Occidental Mindoro, Sablayan	x				ELR 647	*
Platymantis corrugatus	4	Philippines, Occidental Mindoro, Sablayan	x				ELR 661	*
Platymantis corrugatus	5	Philippines, Occidental Mindoro, Calintaan	x				ELR 732	KU 306616
Platymantis corrugatus	5	Philippines, Occidental Mindoro, Calintaan	x				ELR 744	KU 306627
Platymantis corrugatus	6	Philippines, Occidental Mindoro, Magsaysay	x				RMB 4857	*
Platymantis corrugatus	7	Philippines, Quezon, Polillo	x				CDS 1087	KU 300353
Platymantis corrugatus	7	Philippines, Quezon, Polillo	x				RMB 5635	KU 303543
Platymantis corrugatus	7	Philippines, Quezon, Polillo	x	x	x	x	RMB 6251	KU 307576
Platymantis corrugatus	7	Philippines, Quezon, Polillo	x				RMB 6256	KU 307581
Platymantis corrugatus	7	Philippines, Quezon, Polillo	x				RMB 6301	KU 307582
Platymantis corrugatus	7	Philippines, Quezon, Polillo	x				RMB 6302	KU 307583
Platymantis corrugatus	7	Philippines, Quezon, Polillo	x				RMB 6303	KU 307584
Platymantis corrugatus	7	Philippines, Quezon, Polillo	x				RMB 6304	KU 307585
Platymantis corrugatus	8	Philippines, Ilocos Norte, Adams	x				RMB 14184	KU 329764
Platymantis corrugatus	8	Philippines, Ilocos Norte, Adams	x				RMB 14227	KU 329765
Platymantis corrugatus	8	Philippines, Ilocos Norte, Adams	x	x	x	x	RMB 14288	KU 329766
Platymantis corrugatus	9	Philippines, Cagayan, Peablanca	x				ELR 232	*
Platymantis corrugatus	10	Philippines, Cagayan, Gonzaga	x				RMB 14724	KU 330250
Platymantis corrugatus	10	Philippines, Cagayan, Gonzaga	x				RMB 14803	KU 330253
Platymantis corrugatus	10	Philippines, Cagayan, Gonzaga	x				RMB 14871	KU 330254
Platymantis corrugatus	10	Philippines, Cagayan, Gonzaga	x				RMB 14940	KU 330255
Platymantis corrugatus	10	Philippines, Cagayan, Gonzaga	x				RMB 15044	KU 330257
Platymantis corrugatus	10	Philippines, Cagayan, Gonzaga	x				RMB 15114	KU 330260
Platymantis corrugatus	10	Philippines, Cagayan, Gonzaga	x	x	x	x	RMB 15208	KU 330263

Platymantis corrugatus	11	Philippines, Cagayan, Santa Ana	x	x	x	x	RMB 4234	*
Platymantis corrugatus	12	Philippines, Isabela, Palanan	x		x	x	ACD 705	*
Platymantis corrugatus	13	Philippines, Catanduanes, Gigmoto	x				CDS 2458	*
Platymantis corrugatus	13	Philippines, Catanduanes, Gigmoto	x				CDS 2350	KU 308111
Platymantis corrugatus	13	Philippines, Catanduanes, Gigmoto	x	x	x	x	CDS 2464	KU 308229
Platymantis corrugatus	13	Philippines, Catanduanes, Gigmoto	x				CDS 2470	KU 308235
Platymantis corrugatus	14	Philippines, Laguna, Los Banos	x				ACD 6415	*
Platymantis corrugatus	14	Philippines, Laguna, Los Banos	x				ACD 952	*
Platymantis corrugatus	14	Philippines, Laguna, Los Banos	x				CDS 5835	*
Platymantis corrugatus	14	Philippines, Laguna, Los Banos	x				CDS 5839	*
Platymantis corrugatus	14	Philippines, Laguna, Los Banos	x				CDS 5552	KU 330893
Platymantis corrugatus	14	Philippines, Laguna, Los Banos	x				CDS 5554	KU 330894
Platymantis corrugatus	14	Philippines, Laguna, Los Banos	x				CDS 5694	KU 330900
Platymantis corrugatus	14	Philippines, Laguna, Los Banos	x				CDS 5698	KU 330901
Platymantis corrugatus	14	Philippines, Laguna, Los Banos	x				CDS 5719	KU 330902
Platymantis corrugatus	15	Philippines, Aurora, San Luis	x				RMB 10690	KU 322476
Platymantis corrugatus	16	Philippines, Quezon, Tayabas City	x				RMB 3633	*
Platymantis corrugatus	16	Philippines, Quezon, Tayabas City	x				RMB 3634	*
Platymantis corrugatus	16	Philippines, Quezon, Tayabas City	x	x	x	x	RMB 3659	*
Platymantis corrugatus	17	Philippines, Quezon, Atimonan	x				RMB 4069	*
Platymantis corrugatus	17	Philippines, Quezon, Atimonan	x				RMB 4098	*
Platymantis corrugatus	17	Philippines, Quezon, Atimonan	x				RMB 13643	KU 328719
Platymantis corrugatus	18	Philippines, Zambales, Olongapo City	x	x	x	x	RMB 4489	*
Platymantis corrugatus	19	Philippines, Cavite, Ternate	x				ACD 458	*
Platymantis corrugatus	19	Philippines, Cavite, Ternate	x				*	DLSUD 002
Platymantis corrugatus	19	Philippines, Cavite, Cavite	x				*	DLSUD 005
Platymantis corrugatus	19	Philippines, Cavite, Ternate	x				ACD 5658	KU 327577
Platymantis corrugatus	20	Philippines, Batangas, Lipa City	x	x	x	x	ACD 5934	*

Platymantis corrugatus	20	Philippines, Batangas, Lipa City	x				ACD 5958	*
Platymantis corrugatus	21	Philippines, Bulacan, Norzagaray	x				ACD 6030	*
Platymantis corrugatus	21	Philippines, Bulacan, Norzagaray	x				ACD 6031	*
Platymantis corrugatus	21	Philippines, Bulacan, Norzagaray	x				ACD 6039	*
Platymantis corrugatus	21	Philippines, Bulacan, Norzagaray	x				ACD 6040	*
Platymantis corrugatus	21	Philippines, Bulacan, Norzagaray	x				ACD 6122	*
Platymantis corrugatus	21	Philippines, Bulacan, Norzagaray	x				ACD 6123	*
Platymantis corrugatus	21	Philippines, Bulacan, Norzagaray	x				ACD 6183	KU 328871
Platymantis corrugatus	21	Philippines, Bulacan, Norzagaray	x				ACD 6222	KU 328877
Platymantis corrugatus	21	Philippines, Bulacan, Norzagaray	x				ACD 6255	KU 328885
Platymantis corrugatus	22	Philippines, Bulacan, Dona Remedios Trinidad	x				DSM 1883	KU 329022
Platymantis corrugatus	22	Philippines, Bulacan, Dona Remedios Trinidad	x				RMB 13970	KU 329033
Platymantis corrugatus	23	Philippines, Aurora, Maria Aurora	x				RMB 850	*
Platymantis corrugatus	23	Philippines, Aurora, Maria Aurora	x				RMB 971	*
Platymantis corrugatus	23	Philippines, Aurora, Maria Aurora	x				RMB 11900	KU 321956
Platymantis corrugatus	23	Philippines, Aurora, Maria Aurora	x				RMB 12171	KU 321970
Platymantis corrugatus	23	Philippines, Aurora, Maria Aurora	x				RMB 12350	KU 321979
Platymantis corrugatus	23	Philippines, Aurora, Maria Aurora	x				RMB 12166	KU 321980
Platymantis corrugatus	23	Philippines, Aurora, Maria Aurora	x				LJW 0064	KU 321983
Platymantis corrugatus	24	Philippines, Aurora, San Luis	x				RMB 10682	KU 322474
Platymantis corrugatus	24	Philippines, Aurora, San Luis	x	x	x	x	RMB 10683	KU 322475
Platymantis corrugatus	25	Philippines, Camarines Norte, Labo	x				RMB 9598	KU 313698
Platymantis corrugatus	25	Philippines, Camarines Norte, Labo	x				RMB 9631	KU 313699
Platymantis corrugatus	25	Philippines, Camarines Norte, Labo	x				RMB 9780	KU 313704
Platymantis corrugatus	25	Philippines, Camarines Norte, Labo	x	x	x	x	RMB 9790	KU 313706
Platymantis corrugatus	26	Philippines, Camarines Sur, Naga City	x	x	x	x	RMB 3332	*
Platymantis corrugatus	27	Philippines, Albay, Malinao	x	x	x	x	RMB 13686	KU 328720
Platymantis corrugatus	28	Philippines, Albay, Tabaco City	x				RMB 3605	*

Platymantis corrugatus	29	Philippines, Sorsogon, Irosin	x				RMB 3968	*
Platymantis corrugatus	29	Philippines, Sorsogon, Irosin	x				RMB 4008	*
Platymantis corrugatus	29	Philippines, Sorsogon, Irosin	x	x	x	x	RMB 4046	*
Platymantis corrugatus	30	Philippines, Negros Oriental, Valencia	x				RMB 3232	*
Platymantis corrugatus	30	Philippines, Negros Oriental, Valencia	x	x	x	x	RMB 3233	*
Platymantis corrugatus	30	Philippines, Negros Oriental, Valencia	x				RMB 3235	*
Platymantis corrugatus	31	Philippines, Negros Oriental, Bacong	x				CDS 3639	KU 320243
Platymantis corrugatus	31	Philippines, Negros Oriental, Bacong	x				CDS 3640	KU 320244
Platymantis corrugatus	32	Philippines, Siquijor, Siquijor	x		x	x	RMB 1112	*
Platymantis corrugatus	32	Philippines, Siquijor, Siquijor	x				RMB 1113	*
Platymantis corrugatus	32	Philippines, Siquijor, Siquijor	x				RMB 1130	*
Platymantis corrugatus	32	Philippines, Siquijor, Siquijor	x				RMB 15260	KU 331747
Platymantis corrugatus	32	Philippines, Siquijor, Siquijor	x	x	x	x	RMB 15261	KU 331748
Platymantis corrugatus	32	Philippines, Siquijor, Siquijor	x				RMB 15310	KU 331839
Platymantis corrugatus	32	Philippines, Siquijor, Siquijor	x				RMB 15311	KU 331840
Platymantis corrugatus	33	Philippines, Antique, Sibalom	x				GVAG 222	*
Platymantis corrugatus	33	Philippines, Antique, Sibalom	x				GVAG 223	*
Platymantis corrugatus	33	Philippines, Antique, Sibalom	x	x	x	x	GVAG 267	*
Platymantis corrugatus	33	Philippines, Antique, Sibalom	x				GVAG 292	*
Platymantis corrugatus	33	Philippines, Antique, Sibalom	x				R 35	*
Platymantis corrugatus	34	Philippines, Antique, Pandan	x				CDS 1518	*
Platymantis corrugatus	34	Philippines, Antique, Pandan	x				CDS 1525	*
Platymantis corrugatus	34	Philippines, Antique, Pandan	x				CDS 1572	*
Platymantis corrugatus	34	Philippines, Antique, Pandan	x	x	x	x	CDS 1576	KU 304501
Platymantis corrugatus	35	Philippines, Antique, Culasi	x	x	x	x	CDS 1657	KU 304502
Platymantis corrugatus	36	Philippines, Antique, San Remigio	x				RMB 6434	KU 306846
Platymantis corrugatus	36	Philippines, Antique, San Remigio	x				RMB 6574	KU 306852
Platymantis corrugatus	36	Philippines, Antique, San Remigio	x				RMB 6586	KU 306857

Platymantis corrugatus	37	Philippines, Negros Occidental, Silay City	x				CDS 4227	KU 324632
Platymantis corrugatus	37	Philippines, Negros Occidental, Silay City	x				CDS 4420	KU 324633
Platymantis corrugatus	38	Philippines, Leyte, Baybay City	x				ACD 1238	*
Platymantis corrugatus	38	Philippines, Leyte, Baybay City	x	x	x	x	ACD 1239	*
Platymantis corrugatus	38	Philippines, Leyte, Baybay City	x				RMB 4337	*
Platymantis corrugatus	38	Philippines, Leyte, Baybay City	x				RMB 4339	*
Platymantis corrugatus	38	Philippines, Leyte, Baybay City	x				RMB 4340	*
Platymantis corrugatus	38	Philippines, Leyte, Baybay City	x				CDS 3126	KU 311017
Platymantis corrugatus	38	Philippines, Leyte, Baybay City	x				CDS 3249	KU 311019
Platymantis corrugatus	38	Philippines, Leyte, Baybay City	x				CDS 3402	KU 311020
Platymantis corrugatus	38	Philippines, Leyte, Baybay City	x				CDS 3125	KU 311021
Platymantis corrugatus	38	Philippines, Leyte, Baybay City	x				RMB 8951	KU 326238
Platymantis corrugatus	38	Philippines, Leyte, Baybay City	x	x	x	x	RMB 8952	KU 326239
Platymantis corrugatus	39	Philippines, Southern Leyte, Sogod	x				ACD 7188	*
Platymantis corrugatus	40	Philippines, Southern Leyte, Silago	x				ACD 7501	*
Platymantis corrugatus	41	Philippines, Bohol, Bilar	x				CDS 4432	KU 324662
Platymantis corrugatus	41	Philippines, Bohol, Bilar	x				CDS 4433	KU 324663
Platymantis corrugatus	42	Philippines, Bohol, Carmen	x				RMB 1039	*
Platymantis corrugatus	42	Philippines, Bohol, Carmen	x				RMB 1040	*
Platymantis corrugatus	42	Philippines, Bohol, Carmen	x				RMB 1041	*
Platymantis corrugatus	42	Philippines, Bohol, Carmen	x				RMB 1095	*
Platymantis corrugatus	42	Philippines, Bohol, Carmen	x				RMB 1096	*
Platymantis corrugatus	43	Philippines, Eastern Samar, Taft	x				CDS 2820	*
Platymantis corrugatus	43	Philippines, Eastern Samar, Taft	x				CDS 2828	*
Platymantis corrugatus	43	Philippines, Eastern Samar, Taft	x				CDS 2882	*
Platymantis corrugatus	43	Philippines, Eastern Samar, Taft	x				CDS 2956	*
Platymantis corrugatus	43	Philippines, Eastern Samar, Taft	x				CDS 2957	*
Platymantis corrugatus	43	Philippines, Eastern Samar, Taft	x				CDS 1832	KU 304503

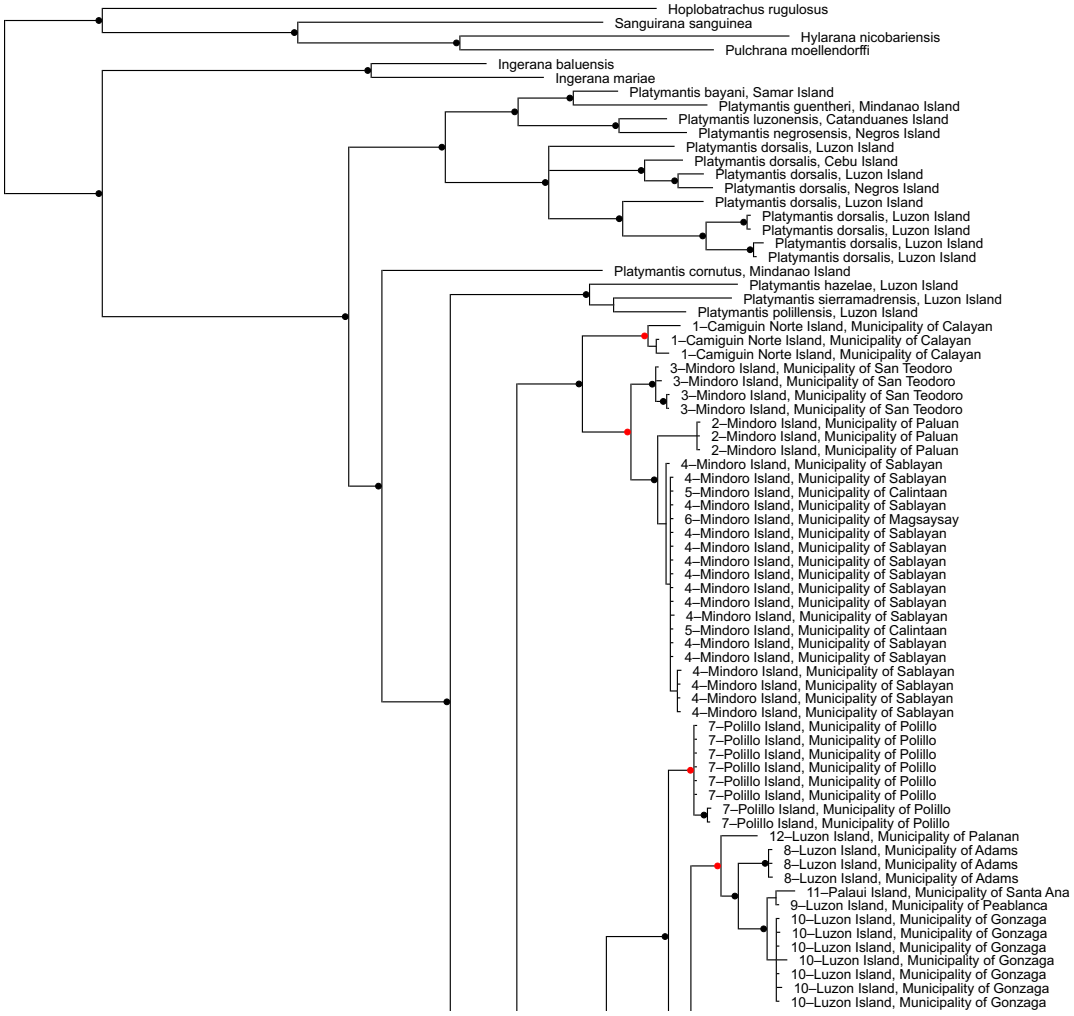
Platymantis corrugatus	43	Philippines, Eastern Samar, Taft	x				RMB 8592	KU 310309
Platymantis corrugatus	43	Philippines, Eastern Samar, Taft	x	x	x	x	RMB 8635	KU 310310
Platymantis corrugatus	43	Philippines, Eastern Samar, Taft	x				RMB 8671	KU 310311
Platymantis corrugatus	43	Philippines, Eastern Samar, Taft	x	x	x	x	CDS 2808	KU 310461
Platymantis corrugatus	43	Philippines, Eastern Samar, Taft	x				CDS 2483	KU 310894
Platymantis corrugatus	43	Philippines, Eastern Samar, Taft	x				CDS 2517	KU 310895
Platymantis corrugatus	43	Philippines, Eastern Samar, Taft	x				CDS 2683	KU 310898
Platymantis corrugatus	43	Philippines, Eastern Samar, Taft	x				CDS 2994	KU 310921
Platymantis corrugatus	43	Philippines, Eastern Samar, Taft	x				CDS 2995	KU 310922
Platymantis corrugatus	43	Philippines, Eastern Samar, Taft	x				CDS 3048	KU 310923
Platymantis corrugatus	44	Philippines, Dinagat Islands, Loreto	x				RMB 8502	KU 310005
Platymantis corrugatus	44	Philippines, Dinagat Islands, Loreto	x	x	x	x	RMB 8503	KU 310006
Platymantis corrugatus	45	Philippines, Agusan del Norte, Remedios T. Romualdez	x				RMB 15844	KU 333301
Platymantis corrugatus	45	Philippines, Agusan del Norte, Remedios T. Romualdez	x	x	x	x	RMB 15845	KU 333302
Platymantis corrugatus	45	Philippines, Agusan del Norte, Remedios T. Romualdez	x				RMB 15932	KU 333303
Platymantis corrugatus	46	Philippines, Agusan del Sur, San Francisco	x				ACD 3849	*
Platymantis corrugatus	46	Philippines, Agusan del Sur, San Francisco	x		x	x	RMB 9444	KU 314062
Platymantis corrugatus	46	Philippines, Agusan del Sur, San Francisco	x	x	x	x	RMB 9445	KU 314063
Platymantis corrugatus	46	Philippines, Agusan del Sur, San Francisco	x	x	x	x	ACD 3951	KU 319560
Platymantis corrugatus	46	Philippines, Agusan del Sur, San Francisco	x				ACD 3975	KU 319564
Platymantis corrugatus	46	Philippines, Agusan del Sur, San Francisco	x				ACD 4026	KU 319565
Platymantis corrugatus	46	Philippines, Agusan del Sur, San Francisco	x				ACD 4192	KU 319573
Platymantis corrugatus	47	Philippines, Camiguin, Mambajao	x				RMB 7953	*
Platymantis corrugatus	47	Philippines, Camiguin, Mambajao	x				RMB 7978	*
Platymantis corrugatus	47	Philippines, Camiguin, Mambajao	x				RMB 7952	KU 309728
Platymantis corrugatus	47	Philippines, Camiguin, Mambajao	x				RMB 7977	KU 309730
Platymantis corrugatus	47	Philippines, Camiguin, Mambajao	x				RMB 8049	KU 309758
Platymantis corrugatus	47	Philippines, Camiguin, Mambajao	x				RMB 8050	KU 309759

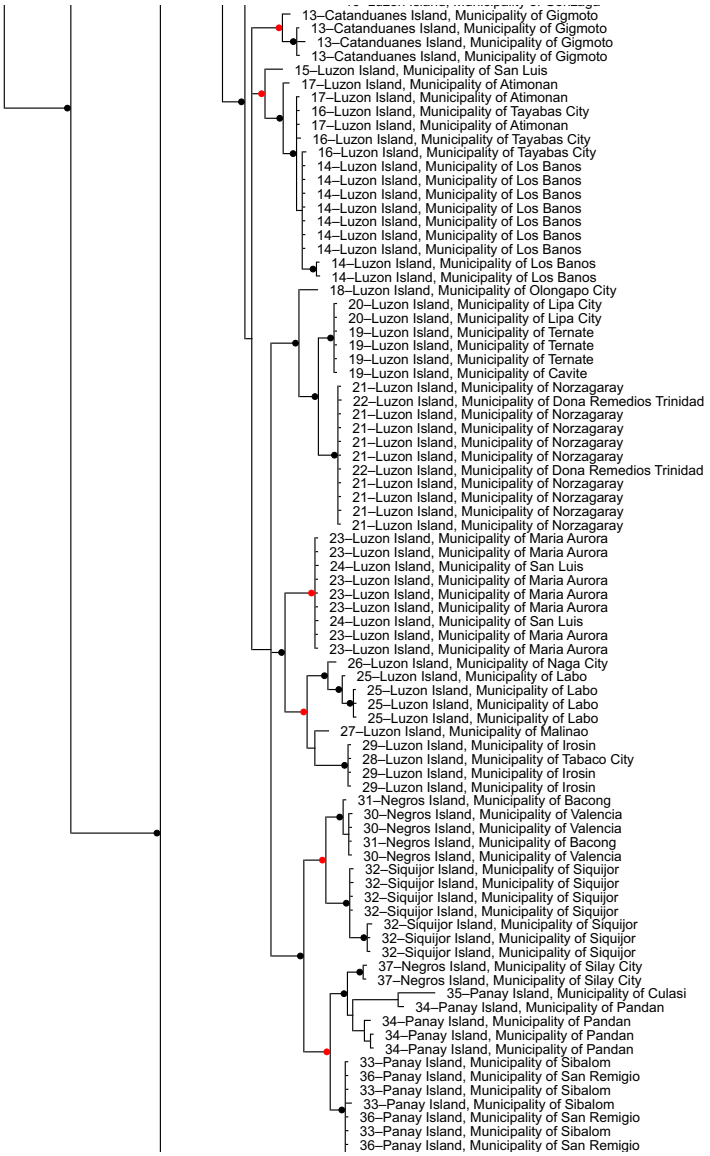
Platymantis corrugatus	47	Philippines, Camiguin, Mambajao	x				RMB 8146	KU 309769
Platymantis corrugatus	47	Philippines, Camiguin, Mambajao	x	x	x	x	RMB 8147	KU 309770
Platymantis corrugatus	48	Philippines, Camiguin, Guinsiliban	x				CDS 105	KU 300351
Platymantis corrugatus	49	Philippines, Sarangani, Kiamba	x	x	x	x	*	CMNH 1548
Platymantis corrugatus	50	Philippines, Davao Oriental, San Isidro	x				ACD 2632	*
Platymantis corrugatus	50	Philippines, Davao Oriental, San Isidro	x				ACD 2657	*
Platymantis corrugatus	50	Philippines, Davao Oriental, San Isidro	x		x	x	ACD 2694	*
Platymantis corrugatus	50	Philippines, Davao Oriental, San Isidro	x				ACD 2701	*
Hoplobatrachus rugulosus	N/A	Thailand		x	x	x	DSM 1700	KU 329114
Hoplobatrachus rugulosus	N/A	Philippines, Laguna, Los Banos	x				ACD 912	*
Hylarana nicobariensis	N/A	Philippines, Aurora, Maria Aurora	x				RMB 2086	*
Ingerana baluensis	N/A	Malaysia	x				*	FMNH 44690
Ingerana mariae	N/A	Philippines, Palawan, Brooke's Point			x	x	RMB 7763	KU 309473
Ingerana mariae	N/A	Philippines, Palawan, Brooke's Point	x				RMB 7803	KU 309518
Platymantis bayani	N/A	Philippines, Eastern Samar, Taft	x	x	x	x	CDS 2986	KU 309257
Platymantis cornutus	N/A	Philippines, South Cotabato, Tampakan	x	x	x	x	ACD 5180	KU 327576
Platymantis dorsalis	N/A	Philippines, Negros Occidental, Negros Occidental	x				ACD 1597	*
Platymantis dorsalis	N/A	Philippines, Aurora, Aurora	x				RMB 849	*
Platymantis dorsalis	N/A	Philippines, Nueva Vizcaya, Quezon	x				ELR 1381	KU 308916
Platymantis dorsalis	N/A	Philippines, Aurora, Maria Aurora	x				RMB 12282	KU 322027
Platymantis dorsalis	N/A	Philippines, Aurora, Maria Aurora	x				RMB 12395	KU 322027
Platymantis dorsalis	N/A	Philippines, Aurora, Maria Aurora	x				RMB 12911	KU 322501
Platymantis dorsalis	N/A	Philippines, Aurora, San Luis	x				RMB 12912	KU 322502
Platymantis dorsalis	N/A	Philippines, Laguna, Los Banos	x	x	x	x	CDS 5841	KU 330904
Platymantis dorsalis	N/A	Philippines, Cebu, Argao	x				RMB 15379	KU 331890
Platymantis guentheri	N/A	Philippines, Agusan del Sur, San Francisco	x				ACD 4170	*
Platymantis hazelae	N/A	Philippines, Nueva Vizcaya, Quezon	x		x	x	RMB 3318	KU 319619
Platymantis luzonensis	N/A	Philippines, Catanduanes, San Miguel	x				CDS 1712	KU 311299

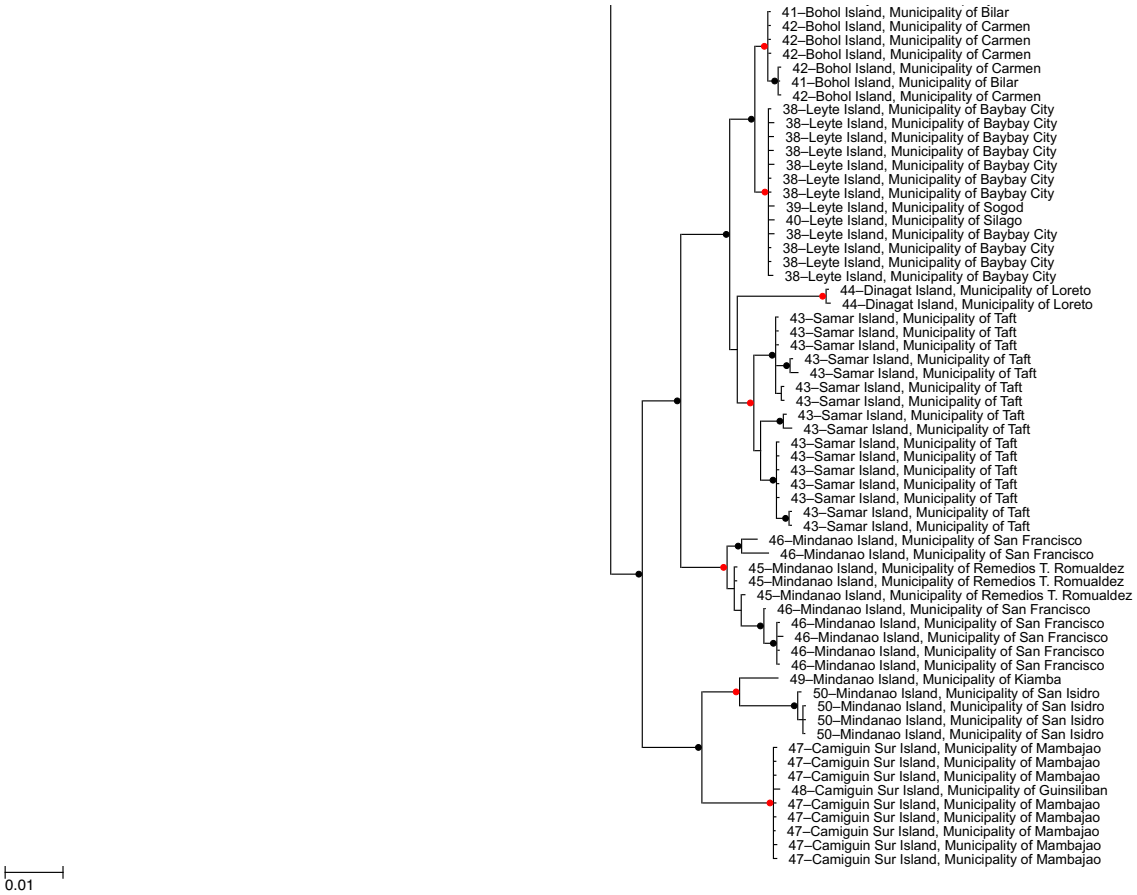
Platymantis negrosensis	N/A	Philippines, Negros Oriental, Valencia	x				CDS 853	KU 309087
Platymantis polillensis	N/A	Philippines, Camarines Norte, Labo	x	x	x	x	RMB 9816	KU 313721
Platymantis sierramadrensis	N/A	Philippines, Aurora, Maria Aurora	x				RMB 12248	KU 322170
Pulchrana moellendorffi	N/A	Philippines, Palawan, Narra	x	x	x	x	RMB 3078	KU 327053
Pulchrana moellendorffi	N/A	Philippines, Palawan, Brooke's Point			x	x	RMB 7807	KU 309312
Sanguirana sanguinea	N/A	Philippines, Palawan, Brooke's Point	x				RMB 3075	KU 329484

APPENDIX 3

Phylogentic tree estimated in MrBayes showing all individuals. Black circles represent nodes with > 0.95 posterior probabilities. Red circles represent nodes with > 0.95 posterior probabilities which were collapsed in Figure 1.




13–Catanduanes Island, Municipality of Gigmoto
13–Catanduanes Island, Municipality of Gigmoto
13–Catanduanes Island, Municipality of Gigmoto
13–Catanduanes Island, Municipality of Gigmoto
15–Luzon Island, Municipality of San Luis
17–Luzon Island, Municipality of Atimonan
17–Luzon Island, Municipality of Atimonan
16–Luzon Island, Municipality of Tayabas City
17–Luzon Island, Municipality of Atimonan
16–Luzon Island, Municipality of Tayabas City
16–Luzon Island, Municipality of Tayabas City
14–Luzon Island, Municipality of Los Banos
14–Luzon Island, Municipality of Los Banos
14–Luzon Island, Municipality of Los Banos
14–Luzon Island, Municipality of Los Banos
14–Luzon Island, Municipality of Los Banos
14–Luzon Island, Municipality of Los Banos
14–Luzon Island, Municipality of Los Banos
14–Luzon Island, Municipality of Los Banos
14–Luzon Island, Municipality of Los Banos
14–Luzon Island, Municipality of Los Banos
18–Luzon Island, Municipality of Olongapo City
20–Luzon Island, Municipality of Lipa City
20–Luzon Island, Municipality of Lipa City
19–Luzon Island, Municipality of Ternate
19–Luzon Island, Municipality of Ternate
19–Luzon Island, Municipality of Ternate
19–Luzon Island, Municipality of Cavite
21–Luzon Island, Municipality of Norzagaray
22–Luzon Island, Municipality of Dona Remedios Trinidad
21–Luzon Island, Municipality of Norzagaray
21–Luzon Island, Municipality of Norzagaray
21–Luzon Island, Municipality of Norzagaray
21–Luzon Island, Municipality of Norzagaray
22–Luzon Island, Municipality of Dona Remedios Trinidad
21–Luzon Island, Municipality of Norzagaray
21–Luzon Island, Municipality of Norzagaray
21–Luzon Island, Municipality of Norzagaray
21–Luzon Island, Municipality of Norzagaray
23–Luzon Island, Municipality of Maria Aurora
23–Luzon Island, Municipality of Maria Aurora
24–Luzon Island, Municipality of San Luis
23–Luzon Island, Municipality of Maria Aurora
23–Luzon Island, Municipality of Maria Aurora
23–Luzon Island, Municipality of Maria Aurora
24–Luzon Island, Municipality of San Luis
23–Luzon Island, Municipality of Maria Aurora
23–Luzon Island, Municipality of Maria Aurora
26–Luzon Island, Municipality of Naga City
25–Luzon Island, Municipality of Labo
25–Luzon Island, Municipality of Labo
25–Luzon Island, Municipality of Labo
25–Luzon Island, Municipality of Labo
27–Luzon Island, Municipality of Malinao
29–Luzon Island, Municipality of Irosin
28–Luzon Island, Municipality of Tabaco City
29–Luzon Island, Municipality of Irosin
29–Luzon Island, Municipality of Irosin
31–Negros Island, Municipality of Bacong
30–Negros Island, Municipality of Valencia
30–Negros Island, Municipality of Valencia
31–Negros Island, Municipality of Bacong
30–Negros Island, Municipality of Valencia
32–Siquijor Island, Municipality of Siquijor
32–Siquijor Island, Municipality of Siquijor
32–Siquijor Island, Municipality of Siquijor
32–Siquijor Island, Municipality of Siquijor
32–Siquijor Island, Municipality of Siquijor
32–Siquijor Island, Municipality of Siquijor
32–Siquijor Island, Municipality of Siquijor
37–Negros Island, Municipality of Silay City
37–Negros Island, Municipality of Silay City
35–Panay Island, Municipality of Culasi
34–Panay Island, Municipality of Pandan
34–Panay Island, Municipality of Pandan
34–Panay Island, Municipality of Pandan
34–Panay Island, Municipality of Pandan
33–Panay Island, Municipality of Sibalom
36–Panay Island, Municipality of San Remigio
33–Panay Island, Municipality of Sibalom
33–Panay Island, Municipality of Sibalom
36–Panay Island, Municipality of San Remigio
33–Panay Island, Municipality of Sibalom
36–Panay Island, Municipality of San Remigio


41–Bohol Island, Municipality of Bilar
42–Bohol Island, Municipality of Carmen
42–Bohol Island, Municipality of Carmen
42–Bohol Island, Municipality of Carmen
42–Bohol Island, Municipality of Carmen
41–Bohol Island, Municipality of Bilar
42–Bohol Island, Municipality of Carmen
38–Leyte Island, Municipality of Baybay City
38–Leyte Island, Municipality of Baybay City
38–Leyte Island, Municipality of Baybay City
38–Leyte Island, Municipality of Baybay City
38–Leyte Island, Municipality of Baybay City
38–Leyte Island, Municipality of Baybay City
38–Leyte Island, Municipality of Baybay City
39–Leyte Island, Municipality of Sogod
40–Leyte Island, Municipality of Silago
38–Leyte Island, Municipality of Baybay City
38–Leyte Island, Municipality of Baybay City
38–Leyte Island, Municipality of Baybay City
38–Leyte Island, Municipality of Baybay City
44–Dinagat Island, Municipality of Loreto
44–Dinagat Island, Municipality of Loreto
43–Samar Island, Municipality of Taft
43–Samar Island, Municipality of Taft
43–Samar Island, Municipality of Taft
43–Samar Island, Municipality of Taft
43–Samar Island, Municipality of Taft
43–Samar Island, Municipality of Taft
43–Samar Island, Municipality of Taft
43–Samar Island, Municipality of Taft
43–Samar Island, Municipality of Taft
43–Samar Island, Municipality of Taft
43–Samar Island, Municipality of Taft
43–Samar Island, Municipality of Taft
43–Samar Island, Municipality of Taft
43–Samar Island, Municipality of Taft
43–Samar Island, Municipality of Taft
46–Mindanao Island, Municipality of San Francisco
46–Mindanao Island, Municipality of San Francisco
45–Mindanao Island, Municipality of Remedios T. Romualdez
45–Mindanao Island, Municipality of Remedios T. Romualdez
45–Mindanao Island, Municipality of Remedios T. Romualdez
46–Mindanao Island, Municipality of San Francisco
46–Mindanao Island, Municipality of San Francisco
46–Mindanao Island, Municipality of San Francisco
46–Mindanao Island, Municipality of San Francisco
46–Mindanao Island, Municipality of San Francisco
49–Mindanao Island, Municipality of Kiamba
50–Mindanao Island, Municipality of San Isidro
50–Mindanao Island, Municipality of San Isidro
50–Mindanao Island, Municipality of San Isidro
50–Mindanao Island, Municipality of San Isidro
47–Camiguin Sur Island, Municipality of Mambajao
47–Camiguin Sur Island, Municipality of Mambajao
47–Camiguin Sur Island, Municipality of Mambajao
48–Camiguin Sur Island, Municipality of Guinsiliban
47–Camiguin Sur Island, Municipality of Mambajao
47–Camiguin Sur Island, Municipality of Mambajao
47–Camiguin Sur Island, Municipality of Mambajao
47–Camiguin Sur Island, Municipality of Mambajao
47–Camiguin Sur Island, Municipality of Mambajao
0.01